REMARKS

Applicant thanks the Examiner for providing initialed copies of PTO-1449 forms. Applicant notes, however, that one reference ("D1", Roberts et al.) on the PTO-1449 form stamped as received on May 5, 2003 by OIPE was not initialed. Applicant respectfully requests that the Examiner provide another copy of this PTO-1449 form, initialed to indicate consideration of the Roberts et al. reference.

Oath/Declaration

A new Oath/Declaration is provided herewith.

Abstract

A further copy of the abstract on a separate sheet filed on 2 February 2001 is provided herewith as page 3 of this amendment. No new matter has been added.

Rejection Under 35 U.S.C. § 103

The Examiner rejected claims 37, 39, 41-46 and 55 under 35 U.S.C. § 103 as unpatentable over Nencioni et al. or Podda et al. in view of Capiau et al., Tamura et al. and Honda et al. Claim 45 is rejected further in view of Halpern et al. Reconsideration of the rejection is requested in light of the following submissions.

The claimed invention is concerned with a method of using a non-toxic double mutant of pertussis toxin as a mucosal adjuvant, i.e. as a substance that stimulates or enhances a protective immune response to an antigen that is co-administered to a mucosal surface with the mutant pertussis toxin. The pertussis toxin has mutations at positions 9 and 129 of the S1 subunit which render it non-toxic by inactivating the ADP-ribosylating enzymatic activity of the native toxin.

It was not obvious that the mutant pertussis toxin as recited in the claims would be an

toxin was likely to be inseparable from its enzymatic activity, and it was therefore expected that inactivating the enzymatic activity of the toxin would also inactivate its adjuvant activity. However, the Applicant actually found the opposite to be the case; he found that, if anything, the mutant pertussis toxin as recited in the claims is a more effective mucosal adjuvant than the wild-type toxin. That was totally unexpected.

Evidence that the adjuvant activity of pertussis toxin was perceived to be likely to be inseparable from its enzymatic activity is provided by Roberts et al. (1995) Infection and Immunity 63, 2100-2108 (which is already of record). See the right column on page 2106 of Roberts et al., where it is stated that:

> *"The mechanism(s) by which PTX exerts adjuvanticity is unknown but is thought to require an enzymatically active S1 subunit"* (emphasis added.)

Roberts et al. goes on to describe one of the experiments which led to the belief that adjuvanticity was thought to require an enzymatically active S1 subunit. In particular, Roberts et al. describes an experiment in which it was shown that heat-killed whole cells of *B. pertussis* strains which had their S1 subunit gene deleted or which had an insertion in S1 resulting in a 90% drop in ADP-ribosylating activity did not enhance the serum antibody response to ovalbumin, whereas killed cells prepared from strains with wild-type PTX genes did (Roberts et al., page 2106, right column).

Further evidence that the adjuvant activity of pertussis toxin was likely to be inseparable from its enzymatic activity is provided by Holmgren et al. (1993) Vaccine 11, 1179-1184. Holmgren et al. is a review of the use of cholera toxin and the B subunit of cholera toxin as an oral-mucosal adjuvant and antigen vector system. Holmgren et al also discusses the heat-labile toxin (LT) from *E. coli*. Pertussis toxin in closely related to both cholera toxin and heat-labile toxin in the sense that all three are bacterial toxins, all three have an AB_5 subunit structure (A = active subunit, B = binding subunit), the A subunits of all three have ADP-ribosylating enzymatic activity and all three have adjuvant activity. Thus, persons skilled in the art believed that what was true of one of the three toxins was generally also likely to be true of the other two.

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Holmgren et al. contains a section entitled "Can adjuvanticity be separated from enterotoxicity?" (see pages 1182-1183) The experiments described in this section of Holmgren et al. clearly suggest that the answer to this question is "no". See, for example, Figure 1 of Holmgren et al., which is reproduced below:

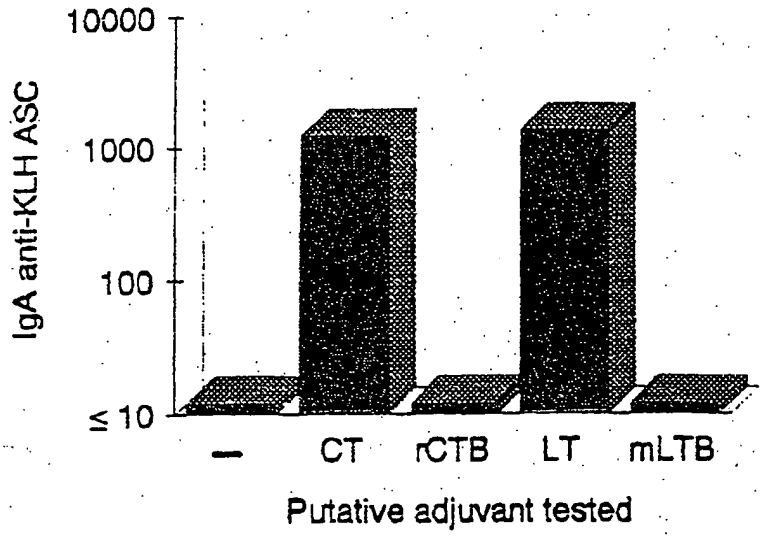

Figure 1 Comparison of the adjuvant effect of cholera toxin (CT), recombinant cholera toxin B subunit (rCTB), *E. coli* heat-labile enterotoxin (LT) and single amino acid residue mutated LT (mLT; contains a single point mutation changing residue 112 in the A subunit from Glu → Lys) on the local immune response in the lamina propria of the small intestine of mice after three peroral immunizations with keyhole limpet haemocyanin (KLH) (2.5 mg per dose) admixed with the putative adjuvants (10 μg per dose) or given alone (—). The frequencies of specific IgA anti-KLH antibody-secreting cells (ASC) per million lamina propria mononuclear cells are shown. (Adapted from Ref. 39)

The results presented in the Figure show that CT (cholera toxin) and LT (heat-labile toxin) are effective adjuvants but that recombinant B subunit of CT ("rCTB") is not an effective

adjuvant. The conclusion that Holmgren et al. reached was that:

> *"This adjuvant activity appears to be closely linked to the ADP-ribosylating action of CT and LT associated with enhanced cyclic AMP formation in the affected cells, and that it may prove difficult to eliminate the enterotoxic activity without loss of adjuvanticity."* (See the abstract of Holmgren et al.)

The Examiner argues that the art had demonstrated that adjuvant activity was independent of enzymatic activity and cites Honda et al. in support of this argument (see the bottom of page 7 of the Official Action). However, for reasons which are explained below, the work of Honda et al. and other similar work was discredited shortly after the publication of Honda et al.

Honda et al. allegedly shows that the B subunit of pertussis toxin is a mucosal adjuvant in the absence of the S1/A subunit. However, it was subsequently shown in the art that this is not correct. In particular, it was subsequently shown that allegedly pure preparations of the B subunit such as that described in Honda et al. were in fact contaminated by very small amounts of active pertussis toxin containing the S1/A subunit and that it was these very small amounts of active pertussis toxin that were responsible for the adjuvant activity. When studies similar to that reported in Honda et al. were repeated using very pure B subunit (e.g., B subunit produced from cells not expressing any active subunit), it was found that there was no adjuvant activity. See, for example, Holmgren et al., page 1182, left column, last complete paragraph, where it is stated that:

> *"Commercial preparations of CTB as used in the 'positive' studies regularly contain 0.1-2% of contaminating CT holotoxin, while we have used a highly purified CTB with no detectable CT (<0.0001%) or recombinant CTB from a genetically CT-deleted V. cholerae strain producing plasmid encoding CTB. On the other hand, when we added 0.1% CT to CTB (0.1 μg to 10 μg as the oral immunising dose) a strong adjuvant effect was observed."*

Similarly, Tamura et al. (1994) Vaccine 12, 419-426 reported that addition of a trace amount of CT to CTB (or LTB) converted a preparation devoid of adjuvant activity into one exhibiting potent adjuvant activity. See the last sentence of the abstract of Tamura et al., which states that:

"These results suggest that CTB (or LTB) containing a trace amount of CT (about 0.1%) can be used practically as a potent adjuvant for nasal vaccination of humans against influenza."

In assessing the art, it is important not to confuse adjuvant activity with antigen activity. As the Examiner will appreciate, adjuvant activity refers to the ability of a substance to stimulate or enhance the immune response against a co-administered substance. In contrast, an antigen merely induces an immune response against itself.

Podda et al. and Nencioni et al. do not teach anything about the adjuvant activity of the mutant pertussis toxin. Podda et al. and Nencioni et al. describe antigen activity of the mutant pertussis toxin, but have nothing whatsoever to do with adjuvant activity.

Applicant maintains that the fact that the mutant pertussis toxin recited in the claims is at least as, if not more, potent than native pertussis toxin as a mucosal adjuvant was unexpected. The Examiner argues that the increase was not unexpected because it had already been suggested by Podda et al. In particular, the Examiner states that "Podda et al. teach that 15 μg of mutant PT-9K/129G induces a humoral response higher than that obtained using 50 or 25 μg of detoxified PT" and concludes from this that the "increase in the response as compared to unaltered PT . . . is not 'unexpected' to the art" (page 9 of the Official Action). However, Applicant respectfully points out that the Examiner has misunderstood Podda et al. Podda et al. is concerned with the antigen activity of PT-9K/129G rather than its adjuvant activity. Furthermore, Podda et al. compares genetically detoxified PT (PT-9K/129G) with chemically detoxified PT (not with native PT). It therefore could and would not have been concluded from Podda et al. that genetically detoxified PT would be a more potent mucosal adjuvant than native PT.

The Examiner argues that the description in the specification fails to indicate the actual amounts of PT and mutant PT-9K/129G administered nasally per dose and that therefore no direct comparison of the two administrations can readily be ascertained (page 9 of the Official Action). Applicant can inform the Examiner that the same amounts of PT and mutant PT-

9K/129G were indeed administered; in both cases, 3 μg was administered. This is evident from the Inventor's publication, Roberts et al.; see the first paragraph of the results section in the right column on page 2101, where it is stated that "3 μg of CT, PTX, or PT-9K/129G" was administered. It is clear that experiments described in Roberts et al are the same as experiments described in the patent application; for example, Figure 3A of Roberts et al. and Figure 7 of the patent application clearly show results of the same experiment.

Finally, Applicant emphasizes that the Examiner should bear in mind that it is notoriously difficult to predict whether a substance will function as an adjuvant, given that the precise manner in which adjuvants work is something of a mystery. It could and would not have been predicted that the non-toxic double mutant of pertussis toxin recited in the claims would be a highly effective adjuvant and would, if anything, be a more effective adjuvant than the native form of the toxin.

In conclusion, the combination of the prior art cited by the Examiner does not provide the elements of Applicant's claimed invention. In particular, Nencioni et al. and Podda et al. teach antigen activity of the mutant pertussis toxin but not any adjuvant activity, and the results of Honda et al. have been discredited with respect to their teachings of adjuvant activity attributable to pertussis toxin B subunit. The teachings of the other cited references do not, in combination, provide the elements missing from Nencioni et al., Podda et al. and Honda et al.

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection of the claims under 35 U.S.C. 103(a).

CONCLUSION

In view of the foregoing amendments and remarks, this application should now be in condition for allowance. A notice to this effect is respectfully requested. If the Examiner believes, after this amendment, that the application is not in condition for allowance, the Examiner is requested to call the Applicant's attorney at the telephone number listed below.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. If there is a fee

occasioned by this response, including an extension fee, that is not covered by an enclosed check, please charge any deficiency to Deposit Account No. 23/2825.

Respectfully submitted,
Mark Roberts, Applicant

John R. Van Amsterdam, Reg. No. 40,212
Wolf, Greenfield & Sacks, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2211
Telephone: (617)720-3500

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